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Changes of ground reaction force and timing variables in the course of habituation of horses to the treadmill

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ABSTRACT

In studies of equine locomotion treadmills and accompanying measuring systems has become a widely used tool. Before any reliable data can be collected horses have to habituate to treadmill locomotion. The aim of the present study was to investigate this process of habituation to an instrumented treadmill by analysing kinetic data and heart rate (HR) at walk and trot and to determine the minimal number of training sessions needed to perform reliable numerical gait analysis. 14 Warmblood horses were assigned to two groups of 7 subjects each, performing either a simple training of 10 min (STG) or a more demanding training of 20 min (ETG). Horses passed 10 consecutive training sessions within 6 days. Before and after each training session, measurements of vertical GRF, contact times, hoof position and HR were made. Separately, for each variable and for each gait, the consecutive measurements of the 2 training regimes were compared with the data of the 10th training session using 2-factor repeated measures ANOVA. The number of sessions needed for habituation was determined accordingly ($P<0.05$). At the trot, objective lameness assessment for clinical use is reliable after a minimum of 4 training sessions, whereas corresponding investigations at the walk demand 4 to 5 training sessions. For longitudinal studies or pre and post treatment investigations, a minimum of 5 sessions is necessary to ensure results that are unbiased from ongoing treadmill experience at both gaits

Keywords:
Horse
Locomotion
Habituation
Treadmill
Kinetics
GRF

Highlights:

- Treadmills have become an essential tool for numerous kinetic and kinematic studies.
- Horses have to habituate to treadmill locomotion before any data can be collected.
- After a maximum of 5 training sessions all observed force, limb timing and spatial variables no longer changed with further treadmill experience.

1. Introduction

Lameness has the highest annual incidence of all medical problems in horses [1]. Consequently, early recognition and reliable examination of lameness is an important and challenging part of daily work in equine veterinary practice. This is done by presenting the horse to the clinician at walk and trot, in a straight line or on a circle and on different surfaces. Another option is to evaluate the horse's gait on a treadmill. Evaluating the gait pattern on a treadmill using constant and reproducible velocities enables a more standardised way of assessment over numerous strides as well as at higher speeds. In the last decades, the treadmill and accompanying measuring systems has become an essential tool for numerous kinematic and kinetic studies in horses worldwide [2-5]. As moving on a treadmill presents a challenge to horses, they have to be habituated to treadmill locomotion before any reliable data can be collected. In humans the process of treadmill familiarisation was described as lasting longer than 15 min at walk [6], ten minutes of walk for clinical use [7] or six minutes at a run in young healthy adults [8]. In dogs, habituation was achieved after one day of treadmill exercise, regardless of the temporal workload per day [9, 10]. In horses however, habituation is described as "a few minutes of rather tense or nervous running" [3] or "one or two exposures to the treadmill at the trot" [11]. Only Buchner et al. [12] has assessed the process of treadmill habituation objectively and revealed that the process of habituation lasted up to 3 sessions at the trot and that for some variables at walk a final steady state was never achieved. These authors also showed that well-habituated horses moved with longer, more regular strides and shorter stride-standardised stance durations (duty factor) compared to novices moving on the treadmill.

At the Equine Department of the Vetsuisse Faculty University of Zurich numerical gait analysis is performed on a treadmill instrumented with a force measuring system [2]. This system allows determination of the vertical ground reaction forces (GRF) of all 4 limbs simultaneously as well as numerous limb timing and positional variables over many consecutive strides. So far, no information is available on how these data depend on the horse's level of habituation to the treadmill. The aims of the present study were:

1) to observe kinetic data during the process of habituating horses to treadmill locomotion over 10 training sessions;

2) to evaluate the effect of 2 training protocols of differing length and complexity;

3) to determine the minimal number of training sessions needed to allow reliable numerical gait analysis and

4) to simultaneously monitor the heart rate as an intrinsic indicator of physical activity but also of stress level.

It is hypothesised that with increasing treadmill experience, horses will show a more relaxed movement characterised by a decrease in stride rate, a pronounced double peak force profile at walk and a lower, more constant heart rate. Furthermore, it was expected that kinetic gait variables would approach asymptotically a stable final value as it was described in previous studies on temporo-spatial variables [10, 12]. A further hypothesis was that the application of a longer and more challenging training protocol, involving changes in gait, velocity and treadmill incline, would speed-up the habituation process and improve gait regularity.

2. Material and methods

2.1 Horses:

Fourteen Swiss Warmblood riding horses were recruited for this study. The 13 geldings and one mare were 4-12 years old (5.5 ± 2.6 y; mean ± standard deviation, s.d.), had a body mass of 490-634 kg (562 ± 44 kg), and a height at the withers of 1.56-1.73 m (1.66 ± 0.06 m). None of them had any previous treadmill experience. All horses were subjected to a thorough orthopaedic examination and were judged clinically sound.

2.2 Experimental design

To habituate the horses to treadmill locomotion, 10 repeated training sessions (T_1 to T_{10}) were performed: twice a day during the first four days and then once a day on the fifth and sixth day. Horses were randomly allocated to two different training protocols (Fig 1). One protocol lasted 10 min and consisted of 5 min walk and 5 min trot; the other protocol lasted 20 minutes, including walking and trotting sequences, halts and changes in treadmill incline and velocity. Horses allocated to the short protocol were named as the simple training group (STG), horses allocated to the longer more demanding protocol as the extended training group (ETG).

On each day, horses were weighed and subjected to a brief clinical and orthopaedic re-examination. Ground reaction forces (GRF) were recorded before (aT_i) and after (pT_i) every training session (i) at the walk and subsequently at the trot (Fig. 1). During every training session heart rate (HR) was recorded continuously.

Immediately before the very first training session, each horse was led onto the treadmill and the treadmill belt was started for a few seconds in order to prepare the horses for the first training session. Thereafter, the treadmill was started again and the first measurement was carried out.

The Animal Health and Welfare Commission of the Canton of Zurich (Switzerland) approved the experimental protocol (TVB-Nr 27 / 2004).

2.3 Data acquisition and analysis

Vertical GRF, contact time and hoof position during ground contact of each limb were measured using a treadmill (Mustang 2200; Kagra-Graber AG, Fahrwangen, Switzerland) instrumented with a force measuring system (TiF, [2]). Data records of 30 s were sampled at 433 Hz, thus including around 24 consecutive strides at walk and 37 at trot. For each stride, various discrete variables were automatically determined using the custom made TiF analysis software (HP2; University of Zurich, Zurich, Switzerland) and further analysed in Excel (Excel; Microsoft Corporation, Redmont, USA). Force and impulse variables were standardised to the horse's body mass. Hoof ground contact timing variables were standardised as percentage of stride duration (%SD), the time of force events (peaks, dip) as percentage of stance duration (%StD) of the respective limb. For each variable, the values from the multiple strides within a record were averaged to a representative value. The corresponding inter-stride standard deviation ($IS_{s.d.}$) served as measure of the variables's variability. As walk and trot are both symmetrical gaits, data of contralateral limbs or corresponding support phases of the half-cycles were pooled. Because left-right asymmetry is an important criterion in lameness diagnostics, an asymmetry index $ASI\,[\%] = 100\%\,(Y_{left} - Y_{right})\,/\,\text{mean}\,(Y_{left}, Y_{right})$ was calculated for most of the variables (Y). ASI is zero for perfect symmetry, negative for a left sided, and positive for a right sided deficit. At this point, it has to be stressed that ASI in this study describes the natural asymmetry as the horses were judged to be non-lame. As in a cohort of horses left and right-sided ASIs would cancel each other to a group-mean of zero the following modified approach was used: For each horse and variable, the ASI for the situation where horses were best accustomed to the treadmill (pT_{10}) served as reference to compare the individual ASI adaptation. For horses that had in the observed variable a negative ASI at pT_{10}, all respective ASI values were sign reversed. In this manner, all horses appeared to have an individual, natural right-sided deficit in that variable, hereafter named as ASIx. This allowed observation of the development of the asymmetry during treadmill habituation using the group-mean ASIx

Heart rate was recorded using a Polar Watch (CS600 Polar Watch; Polar Electro Oy, Kempele, Finnland). The electrodes were fixed under a surcingle on the left chest wall a hand's breadth beneath the withers and 5 cm parasternally. Electrode contact sites were clipped and degreased to lower electrode to skin impedance. Data was processed with Polar Pro Trainer 5 Equine Edition Software (Polar Electro Oy, Kempele, Finnland).

2.4 Statistics

In total, 36 different variables were monitored at the walk and 28 at the trot. For each variable and time point (pT_1 to pT_{10}) the group mean (± s.d.), and for illustrations also the standard error of the mean (s.e.m.), were calculated and the initial mean percentage deviation to pT_{10}, was determined.

The required number of training sessions to achieve reliable measurements was evaluated with the following statistical approach: It was hypothesised that the 10 repeated measurements would not differ from each other and that the training protocol did not influence the variable. These assumptions were tested with the pT_i data, separately for each variable and for each gait, using 2-factor repeated measures ANOVA (RM-ANOVA). Alternatively, it was assumed that the horses were best habituated to treadmill movement after 10 training sessions, a similar guess as used in a former study [12]. Thus, if ANOVA indicated a significant influence of group and/or training session, post-hoc multiple comparison tests against the pT_{10} reference, and between the two training groups, helped to determine the number of required sessions.

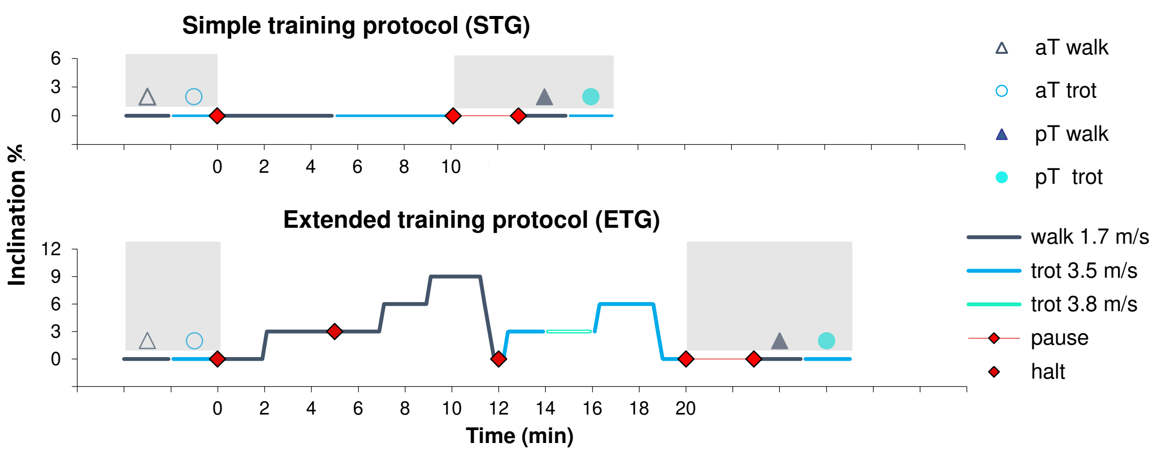


Fig. 1. Illustration of the different training protocols of the two horse groups (STG, ETG). Shown are the time courses of the various sequences regarding gait, speed, and treadmill inclination (y-axis). Intermittently, the treadmill was brought to a brief halt. Additionally indicated are the time points of the measurements

aT and pT of the training session. Before pT horses stepped off the treadmill and the force measuring system was reset to zero. STG, simple training group; ETG, extended training group; aT, ante training; pT, post training.

The question if a further training session significantly improved the variables i.e. if a difference between the measurements before and after a specific session existed, was investigated with a further set of analysis: For each variable, aT_i and pT_i data were submitted to a 2-factor RM-ANOVA, separately for both groups and both gaits. Two effects of interest, either a general ante-post difference (main effect) or a possible $aT_k - pT_k$ difference within the training k^{th} sessions (interaction), were identified with post-hoc multiple comparison tests against the pT_i reference values.

Both analytical procedures were performed for the variable's value, its $IS_{s.d.}$ and, if available, also its ASIx, respectively. For post-hoc tests, Holm-Sidak method for total error control was applied.

Horse's height at the withers, body mass, and age were compared between the two training groups with simple t-tests.

Statistical analysis was done with SigmaStat 3.5 (Systat Software Inc., San Jose, CA, USA). For each test-family, overall error was set at $\alpha = 0.05$ for significance decisions.

3. Results

During a training session, STG horses covered on average a total distance of 2786 ± 142 m (mean ± s.d.) within 18.9 ± 0.3 min (measuring intervals included) whereas ETG horses covered 4229 ± 212 m within 29.7 ± 1.5 min. Surveying all measurement sessions, mean treadmill velocity was 1.72 ± 0.03 m/s at walk and 3.49 ± 0.05 m/s at trot.

3.1 Early reaction to treadmill locomotion

Moving on the treadmill for the very first time was a challenge for each horse. In general, the movement was unsteady and irregular, the strides were short and hasty, and the horses appeared to move in a crouching manner. Horses also showed conspicuous stress symptoms such as snorting and elevated respiratory and heart rates. Compared to the final training session, mean HR at the beginning of the first training session was increased by more than 30 bpm at walk and 40 bpm at trot; in some individuals, HR increased up to twice that amount. The force curves observed at the walk during early training sessions had less prominent force dips and force peaks were temporally closer together compared to later sessions (Fig. 2). In 3 horses, the force peak 1 of the forelimb walking force curve was even intermittently missing, mainly in aT_i and early in the habituation process. Group means of measurements after the 10^{th} training session (pT_{10}; reference) and the percentage difference (Δ%) between the 1^{st} training session (pT_1) and pT_{10} are given in Table 1 (walk) and Table 2 (trot).

3.2 Adaptation of gait variables during treadmill habituation

With increasing habituation, the group means of the values of most variables showed a similar behaviour. Depending on the variable, the values were initially either larger or smaller than the pT_{10} reference and then asymptotically approached that level with further training sessions (Fig. 3). The group standard deviation was always large at the beginning, and then became smaller during the habituation process (see s.e.m. in Fig. 3). Some variables had values from pT_1 to pT_{10} that were not different from each other. However, even for those variables the respective $IS_{s.d.}$ was always initially higher, at least at aT_1. Also $IS_{s.d.}$ of most variables showed an asymptotical decrease with increasing number of training sessions (e.g. Fig. 3B) and was generally small once the horses were habituated. Considering pT_{10} at the walk, stride cycle variables, limb impulses and GRFs, as well as stance durations had inter-stride coefficients of variation ($IS_{c.v.}$) of 1-2.5%, whereas step duration and multi-limb support times as well as times of force peaks had larger $IS_{c.v.}$ of 1-6%. At the trot, $IS_{c.v.}$ at pT_{10} of the mentioned variables was even smaller (1-2%) except for suspension duration (9.5%). For all variables in both gaits, $IS_{c.v.}$ was on average up to 2.2 times higher at aT_1, and still almost 1.3 times higher at pT_1 than pT_{10}.

Horses tended to have an enhanced symmetry (reduced ASIx) for some early sessions compared to the individual natural asymmetry visible after habituation at pT_{10}. At walk, this improved symmetry was significant in many variables (Table 1). At trot only forelimb stance duration and diagonal support duration showed this phenomenon (Table 2).

3.3 Number of training sessions needed to reach habituation

The minimal numbers of training sessions required to achieve habituation, i.e. to measure values that were not statistically different from the reference values, are listed in Table 3 and 4 for walk and trot, respectively. Likewise, the required numbers of sessions for $IS_{s.d.}$ as well as ASIx to adapt are presented in these tables.

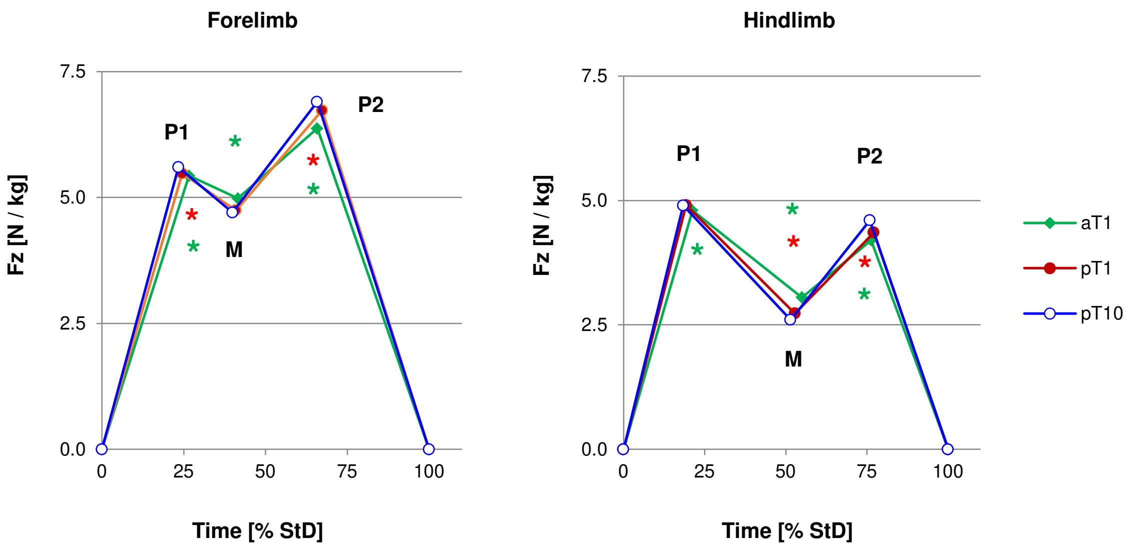


Fig. 2. Sketchs of vertical ground reaction forces (F_z) of fore- and hindlimbs during habituation of the ETG horses to the treadmill before (aT_1) and after the first (pT_1), and after the last (pT_{10}) treadmill training session. Mean values (n=7) of force peaks (P1, P2) and minimum (M); time of occurrence is expressed as

percentage of stance duration (% StD); values are connected with artificial straight lines for illustration purpose. Standard errors of the means (s.e.m.) were smaller than the size of the markers and are therefore not shown. Significant (P<0.05) deviation from the reference (pT$_{10}$) is marked with an asterisk of the respective color.

Table 1

Walk: Group means ± s.d. of the variable's value and ASIx of the STG and the ETG horses (n=7, each), measured after the 10th training session (pT$_{10}$ reference values).

Variabel	Limbs	Units	Value (units)				ASIx (%)			
			Δ%	STG	Δ%	ETG	Δabs	STG	Δabs	ETG
Stride cycle										
stride duration (SD)		s	-2.5^{d}	1.136 ± 0.029	-4.3^{d}	1.194 ± 0.045^{g}				
stride rate		1/min	2.7^{d}	52.8 ± 1.3	4.5^{d}	50.3 ± 1.9^{g}				
stride impulse (I_{SD})		Ns/kg	-2.5^{d}	11.15 ± 0.28	-4.3^{d}	11.71 ± 0.44^{g}				
Impulse & GRF (vertical)										
limb impulse	F	Ns/kg	-4.8^{d}	3.26 ± 0.08	-3.9^{d}	3.38 ± 0.10^{g}	-1.2	1.8 ± 1.0	0.0	2.0 ± 1.0
	H	Ns/kg	0.7	2.31 ± 0.11	-4.7^{d}	2.48 ± 0.12	-1.4	1.9 ± 2.5	-1.9	2.5 ± 2.2
impulse forelimbs / I_{SD}		%I_{SD}	-2.4^{d}	58.5 ± 1.2	0.4	57.7 ± 0.7	--	--	--	--
mean force	F	N/kg	-2.7	4.6 ± 0.1	-0.7	4.6 ± 0.1	0.0	1.9 ± 1.4	0.2	1.2 ± 2.7
force peak 1	F	N/kg	-4.6^{d}	5.6 ± 0.1	-2.2^{d}	5.6 ± 0.3	1.0	1.7 ± 1.0	-3.5^{d}	6.6 ± 5.6^{g}
force minimum	F	N/kg	-2.6	5.0 ± 0.1	0.9	4.7 ± 0.3^{g}	-0.3	3.8 ± 2.1	-2.8	4.1 ± 1.7
force peak 2	F	N/kg	-2.1^{d}	6.8 ± 0.2	-2.5^{d}	6.9 ± 0.2	-2.5^{d}	3.6 ± 2.2	-3.7^{d}	3.4 ± 1.8
mean force	H	N/kg	2.9	3.3 ± 0.1	-0.6	3.3 ± 0.1	0.3	1.7 ± 2.0	2.1	1.7 ± 2.7
force peak 1	H	N/kg	3.1	4.8 ± 0.1	0.1	4.9 ± 0.3	0.0	3.7 ± 2.6	-1.8	5.9 ± 4.9
force minimum	H	N/kg	7.8^{d}	2.7 ± 0.2	5.0^{d}	2.6 ± 0.2	-5.0^{d}	6.2 ± 3.4	-3.5^{d}	5.5 ± 4.6
force peak 2	H	N/kg	-0.6	4.3 ± 0.3	-5.3^{d}	4.6 ± 0.2	-2.8^{d}	5.7 ± 4.9	-5.3^{d}	7.0 ± 3.9
Time (standardised)										
stance duration (duty factor)	F	%SD	0.3	61.9 ± 0.9	1.1^{d}	61.8 ± 1.0	-1.0^{d}	1.6 ± 1.0	-0.9^{d}	1.2 ± 1.0
	H	%SD	0.4	61.4 ± 0.7	0.1	62.1 ± 1.6	-0.4	1.6 ± 1.1	-2.0^{d}	1.7 ± 0.8
diagonal step duration	F→ H	%SD	-2.0	25.5 ± 1.2	2.0	25.9 ± 2.1	-2.0	4.0 ± 2.1	-4.3^{d}	3.6 ± 2.8
tripedal support duration	2F, 1H	%SD	1.3	11.9 ± 0.9	6.1^{d}	11.8 ± 1.0	-4.5^{d}	6.9 ± 6.1	-6.1^{d}	6.6 ± 6.3
ipsilateral support duration	F, H	%SD	-4.8	13.5 ± 1.6	-1.3	14.1 ± 2.8	-2.7^{d}	5.0 ± 2.0	-4.7^{d}	2.7 ± 2.2^{g}
tripedal support duration	1F, 2H	%SD	2.3	11.4 ± 0.7	0.6	12.1 ± 1.6	-1.5^{d}	5.7 ± 2.8	-6.3^{d}	7.2 ± 2.5
diagonal support duration	F, H	%SD	1.7	13.1 ± 1.3	-5.2	12.0 ± 1.8	-1.0	3.9 ± 2.9	-4.6	4.3 ± 2.9
time force peak 1	F	%StD	5.3^{d}	26.9 ± 2.6	5.0^{d}	23.4 ± 3.0^{g}	-2.3	3.6 ± 4.4	-3.1	5.4 ± 3.5
time force minimum	F	%StD	0.6^{d}	42.2 ± 2.7	2.6^{d}	39.9 ± 2.9	-0.4	3.0 ± 1.8	-4.4	5.7 ± 5.2
time force peak 2	F	%StD	0.4	66.3 ± 2.7	2.3	65.8 ± 2.0	-1.4	2.4 ± 1.6	-2.6^{d}	2.0 ± 1.9
time force peak 1	H	%StD	1.4^{d}	18.2 ± 1.6	4.8^{d}	18.4 ± 1.1	-0.1	2.9 ± 1.6	-3.4	3.5 ± 2.5
time force minimum	H	%StD	0.8	52.2 ± 2.0	2.5	51.5 ± 1.3	-1.3^{d}	2.3 ± 1.4	-0.8^{d}	2.7 ± 1.3
time force peak 2	H	%StD	1.6^{d}	77.0 ± 1.1	1.6^{d}	75.9 ± 1.3	0.1	1.8 ± 1.1	-0.7	1.1 ± 0.7
Limb Placement										
stance length	F	m	-2.1^{d}	1.195 ± 0.023	-3.9^{d}	1.270 ± 0.050^{g}	-1.0^{d}	1.5 ± 0.9	-1.3^{d}	1.5 ± 1.5
	H	m	-1.9^{d}	1.174 ± 0.035	-4.2^{d}	1.245 ± 0.039^{g}	-0.1	1.5 ± 0.7	-1.5^{d}	1.2 ± 0.4
contalateral step width	F	m	19^{d}	0.15 ± 0.03	-6.2	0.17 ± 0.01	--	--	--	--
	H	m	7.3^{d}	0.20 ± 0.02	9.9^{d}	0.16 ± 0.03^{g}	--	--	--	--
ipsilateral overreach distance		m	-18	0.26 ± 0.06	-19	0.33 ± 0.07^{g}	--	--	--	--
Heart Rate										
aT_{10}		1/min		59 ± 9		55 ± 5				
aT_1, Δ% aT_1 to aT_{10}		1/min	31^{d}	77 ± 17	40^{d}	78 ± 11				
pT_{10}		1/min		58 ± 5		68 ± 6$^{g\,h}$				
pT_1, Δ% pT_1 to pT_{10}		1/min	24^{d}	72 ± 5	12^{d}	77 ± 6				

Abbreviations: ASIx, asymmetry index; ETG, extended training group; F, forelimb; GRF, ground reaction forces; H, hindlimb; RM-ANOVA, repeated measures ANOVA; s.d. standard deviation; STG, simple training group; %StD, percentage of stance duration, %SD, percentage of stride duration.
ASIx: percentage of left-right asymmetry (see Material and Methods); Δ%: (= 100% (Y_{pT1} - Y_{pT10}) / Y_{pT10}), mean percentage difference for variable (Y) between measurements after the 1st training (pT_1) and pT_{10}; Δabs: mean absolute difference in ASIx between pT_1 and pT_{10}; Heart rate: additionally given are the respective mean values measured before the training sessions (aT_1, aT_{10}) and the matching Δ%.

^{d}: significant (P < 0.05) initial difference Δ% or Δabs

^{g}: significant general group difference in 2-factor RM-ANOVA, denoted here despite that the ANOVA handles all repetitive measurements (pT_1 till pT_{10}) and not only pT_{10}

^{h}: significant aT_{10} vs. pT_{10} difference in heart rate.

3.4 Differences between the two training groups

The biometrical data of the horses of the two groups (STG vs. ETG) did not differ significantly regarding withers height (1.63 ± 0.07 m vs. 1.69 ± 0.04 m; *P* = 0.08), body mass (542 ± 41 kg vs. 582 ± 40 kg; *P* = 0.09), and age (5.0 ± 1.7 years vs. 6.0 ± 3.3 years; *P* = 0.45).

Regarding kinetic and timing variables, some general differences between the two training groups were observed; significances are marked in Table 1 and 2 (factor group of the 2-factor RM ANOVA). The two training groups also behaved differently regarding inter-stride variability. In ETG horses at the trot, $IS_{s.d.}$ of the vast majority of the variables was not different from the respective reference-values after just one training session

(Table 3 and 4). Contrarily, in STG horses at the trot, almost all variables and their respective $IS_{s.d.}$ went through a habituation process lasting several training sessions. $IS_{s.d.}$ at walk and ASIx in both gaits behaved similarly in both groups.

3.5 Comparison between measurements before and after a training session

Individually, aT_i data behaved in a similar asymptotic manner as pT_i. Statistical analysis of the $(a\text{-}p)T_i$ difference identified 3 types of behaviour: 1) no significant a-p difference for any training session (Fig. 4.1; 2) the a-p difference successively decreased with ongoing habituation and vanished no later than one or two sessions after pT_i had adapted to the reference;

Table 2

Trot: Group means ± s.d of the variable's value and ASIx of the STG and the ETG horses (n=7, each), measured after the 10th training session (pT_{10} reference values).

Variable	Limbs	Units	Value (units)				ASIx (%)			
			Δ%	STG	Δ%	ETG	Δabs	STG	Δabs	ETG
Stride cycle										
stride duration (SD)		s	-2.1 ^{d}	0.758 ± 0.044	-3.4 ^{d}	0.785 ± 0.030				
stride rate		1/min	1.9 ^{d}	79.4 ± 4.6	3.6 ^{d}	76.6 ± 2.9				
stride impulse (I_{SD})		Ns/kg	-2.1 ^{d}	7.44 ± 0.43	-3.4 ^{d}	7.70 ± 0.29				
Impulse & GRF (vertical)										
limb impulse	F	Ns/kg	-7.9 ^{d}	2.09 ± 0.12	-3.4 ^{d}	2.15 ± 0.08	0.2	2.2 ± 1.7	-1.3	3.6 ± 3.1
	H	Ns/kg	5.3 ^{d}	1.63 ± 0.11	-3.4 ^{d}	1.70 ± 0.08	-0.7	2.0 ± 0.5	0.8	2.5 ± 1.9 ^{g}
impulse forelimbs / I_{SD}		$\%I_{SD}$	-5.9 ^{d}	56.3 ± 1.2	0.0	56.0 ± 1.0	--	--	--	--
force peak	F	N/kg	-5.3 ^{d}	10.6 ± 0.5	-3.4 ^{d}	10.9 ± 0.3	0.6	1.2 ± 1.3	-0.7	2.3 ± 1.4
	H	N/kg	0.1	9.7 ± 0.4	-4.2 ^{d}	9.9 ± 0.8	0.0	2.1 ± 1.8	0.0	1.8 ± 1.3
Time (standardised)										
stance duration (duty factor)	F	%SD	-1.1	42.3 ± 1.3	2.5 ^{d}	41.4 ± 1.5	-0.5 ^{d}	1.3 ± 0.9	-1.1 ^{d}	0.9 ± 1.1
	H	%SD	5.1 ^{d}	35.7 ± 1.5	3.6 ^{d}	35.9 ± 2.2	-0.8	1.0 ± 0.6	-0.4	1.5 ± 0.6
diagonal support duration	F, H	%SD	4.1 ^{d}	35.7 ± 1.5	2.9 ^{d}	35.8 ± 2.1	-0.3	1.0 ± 0.7	-1.6 ^{d}	1.9 ± 0.6
diagonal suspension duration		%SD	1.9	7.7 ± 1.3	-16 ^{d}	8.5 ± 1.5	-8.5	10.5 ± 5.6	-8.4	15.3 ± 7.1
diagonal advanced placement	F - H	%SD	-54	-1.8 ± 1.1	5.7	-1.0 ± 1.3	--	--	--	--
diagonal advanced completion	F - H	%SD	-28 ^{d}	4.8 ± 1.5	-6.8	4.4 ± 1.0	--	--	--	--
time force peak	F	%StD	6.4 ^{d}	47.8 ± 2.0	1.6	47.5 ± 2.5	0.4	1.3 ± 1.3	-0.5	1.8 ± 0.8
	H	%StD	5.4 ^{d}	49.5 ± 0.7	0.8	49.7 ± 0.7	-1.0	1.4 ± 1.2	-1.4	1.6 ± 0.9
contralateral step duration	F	%SD	--	--	--	--	-1.4	2.1 ± 1.8	-3.1	3.7 ± 1.8
	H	%SD	--	--	--	--	0.4	1.9 ± 1.6	-1.1	1.8 ± 1.0
Limb Placement										
stance length	F	m	-1.9 ^{d}	1.079 ± 0.029	-1.0	1.110 ± 0.058	0.0	1.4 ± 0.5	-0.2	1.2 ± 0.9
	H	m	5.4 ^{d}	0.899 ± 0.021	0.7	0.939 ± 0.059	-0.6	1.6 ± 1.3	-0.3	1.4 ± 1.0
contralateral step width	F	m	18 ^{d}	0.14 ± 0.03	-11 ^{d}	0.16 ± 0.03	--	--	--	--
	H	m	8.5	0.19 ± 0.03	0.1	0.12 ± 0.04 ^{g}	--	--	--	--
diagonal mid-stance length	F - H	m	0.5	1.19 ± 0.03	-0.7	1.22 ± 0.03	-1.1	1.5 ± 1.3	-1.3	2.0 ± 1.4
ipsilateral overreach distance	F - H	m	-17	0.05 ± 0.07	-43 ^{d}	0.07 ± 0.05	--	--	--	--
Heart Rate										
aT_{10}		1/min		94 ± 11		86 ± 6				
aT_1, Δ% aT_1 to aT_{10}		1/min	19 ^{d}	111 ± 13	35 ^{d}	116 ± 16				
pT_{10}		1/min		86 ± 8		91 ± 8				
pT_1, Δ% pT_1 to pT_{10}		1/min	34 ^{d}	115 ± 25	15 ^{d}	105 ± 12				

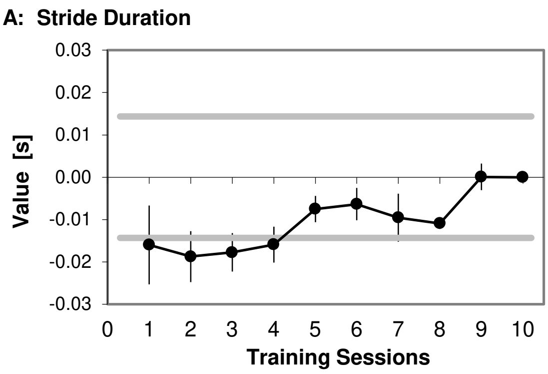
Abbreviations: ETG, extended training group; F, forelimb; GRF, ground reaction forces; H, hindlimb; s.d., standard deviations; %SD, percentage of stride duration; %StD, percentage of stance duration; STG, simple training group.
Diagonal support duration: double contact duration of the diagonal limb pair; Diagonal suspension duration: whole body aerial duration; Diagonal advanced placement: positive time difference by which the diagonal hind limb impacted before the fore limb; Diagonal advanced completion: positive time difference by which diagonal hind limb lifted off before the forelimb. For further explanations, refer Table 1.

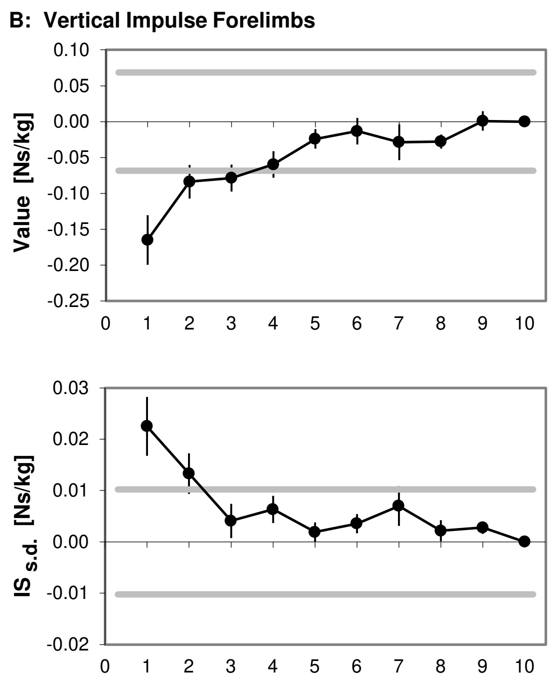
3) the a-p difference persisted, either much longer than the number of sessions required to achieve steady state post session values, or for all sessions (Fig. 4.2; 4.3). Due to the asymptotical behaviour of most variables, aT_i values deviated from those of pT_i, if at all, in the same direction as pT_1 deviated from pT_{10}. The number of training sessions required to reach insignificant a-p differences are listed in Table 3 and Table 4 in brackets.

Long lasting or permanent a-p differences occurred mainly at the walk and for a larger number of variables in the ETG. The same observations were also made regarding the $IS_{s.d.}$. In both gaits, ETG horses generally showed shorter strides at aT_i than pT_i, but no such difference occurred in the STG. In both groups at the walk, hindlimb stance duration was prolonged and forelimb force minima were less developed (i.e. higher, Fig. 2) at the beginning of a training session. Changes within the 2- and 3-limb support phases are presented in Figure 4. At the trot, forelimb stance length and diagonal midstance length were longer, but ipsilateral overreach distance was shorter at aT_i than pT_i. Forelimb peak vertical force had no a-p difference for the first two training sessions (during which pT_i adapted to the reference) but in all succeeding training sessions, peaks were higher at aT_i than pT_i.

3.6 Heart Rate

Heart rates were distinctly elevated during the very first contact with the treadmill. Group mean HR for aT_1 and pT_1 measurements and respective reference values (aT_{10}, pT_{10}) are given in Tables 1 and 2. Similar to other variables, HR approached the reference value asymptotically with increasing number of trainings (Fig. 5). At the trot, HR of both groups developed to similar reference levels in both aTi and pTi measurements. In contrast, at walk only HR measured ante-training adapted to similar levels for the two groups. The post-training rates in ETG approached a reference level, which was about 10 bpm higher than that of the STG. From the 5th training session, HR in ETG also showed an a-p difference of about 12 bpm that persisted to T_{10} (Fig. 5). Regarding aT_i measurements, the reduction of HR to levels that indicated no additional treadmill associated stress was achieved in both gaits after 2 (STG) and 3 (ETG) training sessions, respectively (Table 3 and 4).





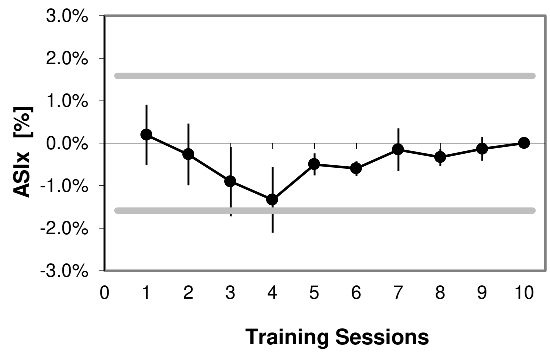


Fig. 3. Changes of selected variables over the course of treadmill habituation during 10 consecutive training sessions in STG horses at the trot (n = 7). Group-mean differences (±s.e.m.) between the measurements after the training sessions (pT_i) and the reference (pT_{10}). A: Values of stride duration, B: Values of fore limb impulses, their mean $IS_{s.d.}$ and the lefteright ASIx. Ninety-five percent tolerance interval for the group-mean differences (gray lines) calculated from the mean square of the residuals for pure repeated measurements. Mean square of the residuals was determined by 1-factor RM-ANOVA. ASIx, asymmetry indices; aT, ante training; $IS_{s.d.}$, interstride standard deviations; pT, post training; RM-ANOVA, repeated measures ANOVA; s.e.m., standard error of the means; STG, simple training group.

4. Discussion

One of the most important prerequisites for successful gait analysis is that the subject is well adapted to the measuring conditions in order to show a true representation of its individual gait pattern. Treadmill movement is especially challenging for animals due to the pre-set velocity and the unfamiliar "movement on spot" in a laboratory setting. In longitudinal studies or pre and post treatment investigations, it has to be ensured that changes in gait pattern cannot be attributed to increasing treadmill experience. Thus, a certain number of training sessions on the treadmill are essential. The same principle applies to overground force plate measurements, where a certain degree of habituation is needed to ensure standardisation of the procedure [13].

4.1 Adaptation of gait variables during habituation to treadmill exercise

Adaptation to treadmill exercise mainly involves a gait transformation from an unsteady and tense to a comfortable and relaxed movement pattern. The present study revealed some of the horse's strategies to deal with this initial insecurity: 1) reducing the vertical dynamics, 2) "squaring" the base of support, 3) enhancing ground contact by reducing instable support phases, and 4) symmetrising left and right half cycles of a stride. Compared to the fully habituated condition, horses that first mount the treadmill improved stability by subtle changes in their movement pattern as follows:

In both gaits, horses shortened their strides, thus reducing the vertical stride and limb impulses which results in a less dynamic gait. Shortening of stride duration at walk (STG: -28 ms; ETG: -51 ms) was comparable to earlier observations (-40 ms, estimated from the graphical presentations of Buchner *et. al.* [12]). A similar strategy to reduce the vertical dynamics by shortening the stride, was observed in horses with mild to moderate weight bearing lameness [18, 19] and in dogs showing clinical and radiographical signs of hip dysplasia [20]. Some horses (mainly in the STG) additionally redistributed weight towards the hindlimbs. This observation is, to our knowledge, not previously described and therefore requires further investigation. Treadmill habituated horses had distinctly different contralateral step widths in fore- and hindlimbs. However, at the beginning of the habituation process, horses matched the step width of both limb pairs, by either widening or narrowing the steps of the fore or the hind limbs, towards a more "square" limb placement. This was not fully consistent with a previous study, which solely reported a general widening of steps [12]. Wider steps may improve lateral stability [21]. However, we also hypothesise that with a "square" limb placement, the centre of gravity may move more evenly in the frontal plane, i.e. with similar but mirror-imaged, lateral excursions during each half cycle, regardless of the number of limbs in support.

At the walk, predominantly at the beginning of the training sessions (aT_i values), horses prolonged the stable tripedal support phases at the expense of the highly instable lateral double support. This was achieved by increasing hindlimb stance durations, which led to a longer double support phase of the hindlimb pair. Prolonged double support phases are observed also in elderly people improving gait stability [21, 22]. For the same limb support phases an increased symmetry between the left and right half cycle was maintained. Only minor changes in stance duration occurred in the pT_i measurements, thus changes in mean GRFs followed the changes in impulse distribution between forehand and hindquarters (forelimb impulse /I_{SD} in Table 1) [14]. With increasing treadmill experience, the force profile at walk developed a more pronounced force dip. In the forelimbs this was caused by the increase in height of the double force peaks and in the hindlimbs by a reduction in the force minima values (Fig. 2). The latter might originate, at least partly, from enlarged stance lengths in well-habituated horses, causing greater vertical upwards acceleration of the caudal trunk region and therefore pronounced unloading at midstance similar to the changes observed with increasing walking velocity[23]. In the forelimbs

Table 3

Walk: Number of training sessions required to achieve a steady state level of the variable's value, $IS_{s.d.}$, and ASIx for the STG and the ETG horses (n=7, each).

Variable	Limbs	Units	Value		$IS_{s.d.}$		ASIx	
			STG	ETG	STG	ETG	STG	ETG
Stride cycle								
stride duration (SD)		s	4	4 (*)	3 (3)	3 (*)		
stride rate		1/min	4	4 (*)	3 (3)	3 (*)		
stride Impulse (I_{SD})		Ns/kg	4	4 (*)	3 (3)	3 (*)		
Impulse & GRF (vertical)								
limb impulse	F	Ns/kg	3	3 (*)	4 (3)	4 (6)	1	1
	H	Ns/kg	**1**	**4** (*)	3 (*)	3 (6)	1	1
impulse forelimbs / I_{SD}		$\%I_{SD}$	**2**	**1**	4 (3)	4 (3)	--	--
mean force during stance	F	N/kg	2	2 (*)	4 (3)	4 (6)	1	1
force peak 1	F	N/kg	2 (*)	2	3 (*)	3 (5)	**1**	**4**
force minimum	F	N/kg	1 (*)	1 (*)	3 (*)	3	1	1
force peak 2	F	N/kg	2	2 (6)	**3** (*)	**4** (2)	4	4 (2)
mean force during stance	H	N/kg	**1**	**4** (*)	3 (*)	3 (6)	1	1
force peak 1	H	N/kg	1	1	3 (*)	3 (9)	1	1
force minimum	H	N/kg	4	4 (3)	2 (*)	2 (6)	2	2
force peak 2	H	N/kg	**1** (2)	**5** (*)	4 (5)	4 (*)	4	4
Time (standardised)								
stance duration (duty factor)	F	%SD	**1**	**2**	3 (2)	3 (6)	5	5
	H	%SD	1 (*)	1 (*)	5 (*)	5 (6)	**1**	**5** (*)
diagonal step duration	F→ H	%SD	1 (*)	1 (*)	3 (4)	3 (6)	**1**	**5**
tripedal support duration	2F, 1H	%SD	**1**	**2**	3 (3)	3 (6)	4	4 (2)
ipsilateral support duration	F, H	%SD	1 (*)	1 (*)	3 (*)	3 (6)	3	3 (2)
tripedal support duration	1F, 2H	%SD	1 (*)	1 (*)	1 (*)	1 (2)	3	3
diagonal support duration	F, H.	%SD	1 (*)	1 (6)	3 (*)	3 (6)	1	1
time force peak 1	F	%StD	3	3 (6)	4	4 (*)	1	1
time force minimum	F	%StD	3	3 (*)	1 (*)	1 (6)	1	1
time force peak 2	F	%StD	1 (*)	1 (*)	3 (4)	3 (6)	**1**	**3**
time force peak 1	H	%StD	5	5 (*)	1 (2)	1 (2)	1	1
time force minimum	H	%StD	1 (3)	1 (*)	4 (*)	4 (6)	3	3 (*)
time force peak 2	H	%StD	2	2	1 (4)	1 (3)	1	1
Limb Placement								
stance length	F	m	4	4 (*)	1 (3)	1 (6)	5	5
	H	m	4	4 (*)	3 (3)	3 (6)	**1**	**9** (! 5)
contralateral step width	F	m	**2** (*)	**1** (*)	1 (9)	1 (4)	--	--
	H	m	2	2	2 (9)	2 (3)	--	--
ipsilateral overreach distance		m	1	1 (*)	3 (*)	3 (*)	--	--
lateral stance start position	F	m	--	--	1 (*)	1 (2)	--	--
longitudinal stance start position	F	m	--	--	1	1 (2)	2	2
Heart Rate								
ante training, aT_i		1/min	2	3				
post training, pT_i		1/min	3	2 (! 5)				

Abbreviations: ASIx, asymmetry index; ETG, extended training group; F, fore limb; GRF, ground reaction forces; H, hind limb; RM-ANOVA, repeated measures ANOVA; s.d. standard deviation; STG, simple training group; %StD, percentage of stance duration, %SD, percentage of stride duration.
Number of training sessions the variable's post training measurement (pT_i) needed for not being statistically different from the one after the 10^{th} training (pT_{10}, reference). Numbers in plain font denote that the required number of training sessions are independent of the training protocol/group (main influence of factor training), whereas the numbers in bold font indicate that habituation depends on both, training number and protocol (interaction).
In parentheses indicated are (k): Number of sessions required until no difference was observed between aT_i - pT_i of a respective training session; (*): General ante-post difference over all sessions (main effect); (! k): Continuous ante-post difference starting from T_k, until T_{10}; Without parentheses: no ante-post difference in any training.
For further explanations, refer Table 1.

this inverted pendulum mechanism is less distinct presumably because shoulder forward-backward rotation in the horizontal plane, which is described as being 3 times larger than at the hip [24], contributes partly to the stance length prolongation.

At the trot, horses also reacted to the insecure situation by prolonging the diagonal double support phase. Again, this was achieved by longer stance durations in the hindlimbs, which improved the support of the centre of mass. Horses in the ETG additionally prolonged forelimb stance duration that resulted in a reduced suspension phase, reduced vertical dynamics and smaller peak forces in all limbs for pTi observations in the insecure state, and at the start of every training session (aTi), respectively. In the STG, forelimb peak forces were initially reduced only due to a notable impulse shift to the hind limbs. The prolongation of hindlimb stance duration counteracted the impulse shift towards caudal in the STG, which led to the hindlimb peak vertical forces remaining constant for all observations. The observed high degree of left-right symmetry of the limb stance durations and the diagonal stances might also be viewed as part of the effort to stabilise the movement during initial insecurity.

4.2 Number of training sessions required to reach habituation

The crucial question of when habituation to the treadmill has been achieved depends on the selected decision criteria. With regards to numerical gait analysis, the gait variables themselves can be deemed to set the point where a steady state movement pattern is reached. For the statistical decision-process in the present study, a RM-ANOVA was chosen, as this is also the procedure, which generally serves to identify treatment effects in longitudinal studies. Furthermore, decisions on the reproducibility of gait pattern was preferably relied on pT_i measurements as in any clinical and experimental investigation horses are warmed up at least some minutes to achieve not only a regular gait but also the compliance of the animal.

Table 4

Trot: Number of training sessions required to achieve a steady state level of the variable's value, $IS_{s.d.}$, and ASIx for the STG and the ETG horses (n=7, each).

Variable	Limbs	Units	Value		$IS_{s.d.}$		ASIx	
			STG	ETG	STG	ETG	STG	ETG
Stride cycle								
stride duration (SD)		s	**5**	**3** (*)	**3** (2)	**1** (2)		
stride rate		1/min	**5**	**3** (*)	**3** (2)	**1** (2)		
stride Impulse (I_{SD})		Ns/kg	**5**	**3** (*)	**3** (2)	**1** (2)		
Impulse & GRF (vertical)								
limb impulse	F	Ns/kg	4 (3)	4	**3** (*)	**1** (2)	1	1
	H	Ns/kg	**2** (3)	**3** (*)	**2**	**1** (2)	1	1
impulse forelimbs / I_{SD}		%I_{SD}	**2** (3)	**1**	**3** (3)	**1** (2)	-	-
force peak	F	N/kg	3 (*)	3 (9)	1 (2)	1 (2)	1	1
	H	N/kg	**1** (3)	**4**	**2**	**1** (2)	1	1
impulse of diagonals			-	-	-	-	1	1
Time (standardised)								
stance duration (duty factor)	F	%SD	**1**	**2**	**3** (2)	**1** (2)	4	4 (3)
	H	%SD	4	4	**2** (2)	**1** (2)	1	1 (*)
diagonal support duration	F, H	%SD	4	4	**3** (2)	**1** (2)	2	2
diagonal suspension duration		%SD	**1** (3)	**4**	**2** (2)	**1** (2)	1	1
diagonal advanced placement	H - F	%SD	1 (3)	1	1 (2)	1	-	-
diagonal advanced completion	F - H	%SD	**2**	**1**	1	1	-	-
time force peak	F	%StD	**3**	**1**	2 (2)	2 (2)	1	1
	H	%StD	**2** (2)	**1** (2)	3 (3)	3 (2)	1 (4)	1
contralateral step duration	F	%SD	-	-	-	-	1	1 (*)
	H	%SD	-	-	-	-	1	1
Limb Placement								
stance length	F	m	3 (*)	3 (! 3)	1 (2)	1 (2)	1	1
	H	m	**2** (4)	**1** (2)	**2** (3)	**1** (2)	1	1
contralateral step width	F	m	**2**	**4**	2 (3)	2 (2)	-	-
	H	m	1	1	**2** (3)	**1** (2)	-	-
diagonal mid-stance length	F - H	m	1 (*)	1 (7)	1 (4)	1 (2)	1	1
ipsilateral overreach distance	F - H	m	**1** (*)	**4** (! 2)	1 (2)	1 (2)	-	-
lateral stance start position	F	m	-	-	1 (*)	1 (2)	-	-
longitudinal stance start position	F	m	-	-	1	1 (2)	1 (7)	1
Heart Rate								
ante training, aTi		1/min	2	3				
post training, pTi		1/min	3	3				

Abbreviations: ASIx, asymmetry index; ETG, extended training group; F, fore limb; GRF, ground reaction forces; H, hind limb; RM-ANOVA, repeated measures ANOVA; s.d. standard deviation; STG, simple training group; %StD, percentage of stance duration, %SD, percentage of stride duration.
For further explanations, refer Table 2 and 3.

Stride duration, one of the most important variables characterising the gait pattern, reached steady state values after 3-5 training sessions in both walk and trot. Within this training period, the limb impulses, the limb positioning during stance (reflected by step width and stance lengths) as well as the stress level (quantified by heart rate) stabilised at both gaits and under both training regimes. Obviously, at the walk relative stance durations, as well as the bipedal and tripedal limb support phases habituated faster (1-2 sessions), regardless of the still ongoing prolongation of the stride cycle. Because stance duration is, beside the fore-hind impulse distribution, a dominant factor influencing the vertical force magnitudes [14], forelimb GRFs adapted equally fast. However, in the hindlimbs extremes of the force profile and their time of occurrence needed up to 5 sessions to habituate as stance length, which causally shapes the dip in the walking force curve, paralleled the changes in stride duration. In contrast, at trot, stance and stride duration of the hindlimbs stabilised simultaneously but the process lasted up to 4 training sessions that consistently influenced all timing variables involving the hindlimbs as well as hindlimb peak forces.

A further criterion under consideration as a marker for habituated treadmill movement is an inter-stride variability that no longer changes with further training sessions. There is evidence, that a horse moving comfortably and without stress shows a regular gait with small $IS_{s.d.}$ of the variables [12]. In the present study most variables' $IS_{s.d.}$ reached a steady state sooner than their respective value at the trot, and for the stride cycle variables at the walk. In contrast, at the walk $IS_{s.d.}$ of the timing variables reached steady state later than the value itself; exceedingly late for the hindlimb stance duration. The observation
that right from the beginning horses' position on the treadmill at the walk was extremely stable ($IS_{s.d.}$ of lateral and longitudinal stance start position in Table 3) led to the assumption that modulating hind stance duration reflects the attempt of the horses to maintain the treadmill position. This attempt is visible mainly in the hindlimbs, because they supply a large part of the propulsive impulse [15] [16]. As expected, at the trot (a highly symmetrical and automatized gait) low $IS_{s.d.}$ measurements were soon achieved; in the ETG within 1-2 sessions. This might be accounted by the longer and more demanding training sessions in this group leading to a highly regular movement pattern.

One might expect that well habituated horses would show for a specific variable similar value before and after a training session; something which could be additionally used as criterion for successful treadmill habituation. This was obviously not the case. Contrarily, long lasting or permanent a-p differences occurred at the walk and for the majority of variables in the ETG. This indicates, that within each further training session horses underwent a small re-habituation process resulting in a more relaxed gait with smaller inter-stride variation at the end of the session, irrespective of the status of treadmill adaptation.

The maximally 3-5 training sessions needed to achieve treadmill habituated movement determined in the present study contrasts partly with the only comparable study in horses, which investigated changes of temporal and kinematic variables of limbs and body [12]. Those authors reported that at walk, stride duration never reached a steady state over the course of 9 sessions each

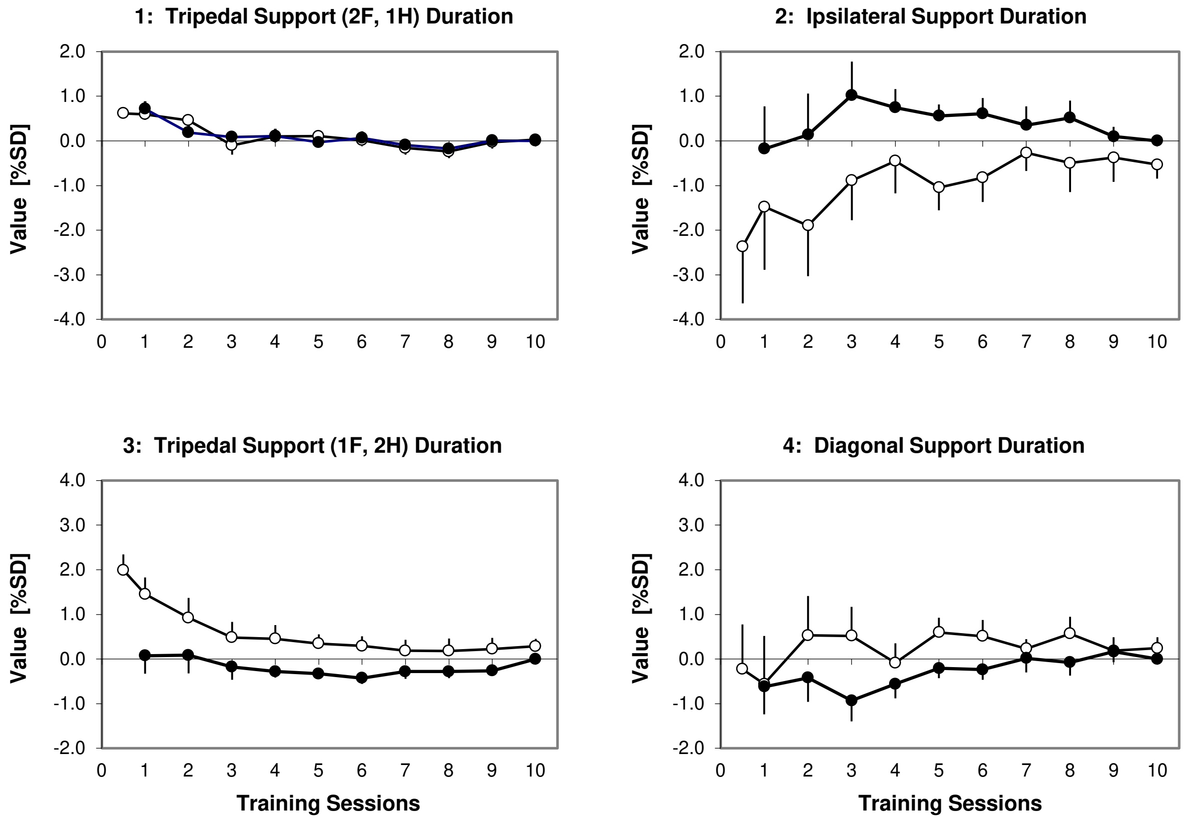


Fig. 4. Changes of the four multilimb support durations within a half cycle during 10 consecutive training sessions in ETG horses (n=7) at the walk, given as %SD. Group mean differences (±s.e.m.) of values before (open circles) and after (closed circles) the training session to the reference (pT_{10}). Also shown is the value for aT_0, the very first treadmill experience, plotted at abscissa 0.5. In limb support phases 2 (Fig. 4.2) and 3 (Fig. 4.3) persisting ante-post differences were observed until the last session. At the beginning of each training session, the most unstable ipsilateral-support phase was shortened and compensated by the prolonged succeeding tripedal-support phase. Refer to Tables 3 and 4 regarding significance and ante-post differences. aT, ante training; F, fore limb; H, hind limb; ETG, extended training group; %SD, percentage of stride duration; s.e.m., standard error of the means; pT, post training.

lasting 10 min, whereas at the trot the steady state was achieved after the first session. In the present study however, stride duration stabilised in both gaits and after about the same number of training sessions. Consequently, similar discrepancies were found when comparing the pro-retraction limb angles monitored in the earlier study with the stance lengths measured in the present study (both variables describe the limb movement angulation and depend on stride duration). Relative stance duration was reported to habituate generally very fast (1 session), taking longest in the hindlimbs at the trot (3 sessions) [12]; a pattern similar to the one observed in the present study. However, a predominantly more rapid habituation of forelimb variables compared to the ones of the hindlimb, as claimed in the earlier work, was not observed in this study; adaptation of some variables even occurred faster in the hind- than in the forelimb.

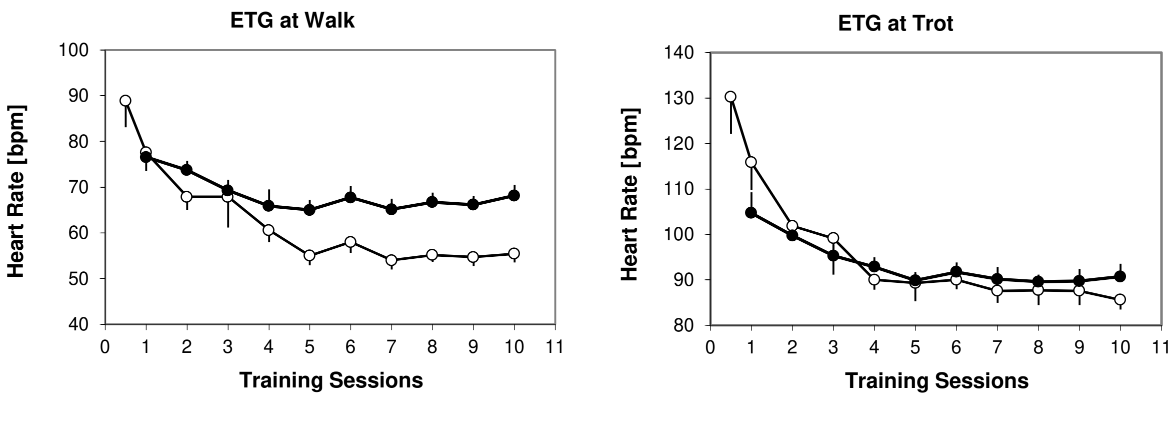


Fig. 5. Heart rates of ETG horses (n=7) over the course of habituation to the treadmill. Shown are the group means (±s.e.m.) of the measurements before (open circles) and after (closed circles) the training sessions, both at walk and trot. Regarding significant differences to the reference (T_{10}) or ante-post differences refer Tables 3 and 4. ETG, extended training group; s.e.m., standard error of the means.

Corresponding to the earlier work, $IS_{s.d.}$ of most variables adapted to reference $IS_{s.d.}$ sooner at trot than at walk. However, with 5 sessions, $IS_{s.d.}$ of stance durations at the walk in both training groups needed much longer to adapt than reported previously [12].

The contrasts between the two studies might originate partly from methodological differences of which the decision method used to conclude when habituation was achieved might be the most influential. In the previous study [12] a threshold for each variable was used that relied on the mean inter-stride variation of that variable determined at the end of the trial series. In the present study, habituation was deemed successful as soon as the observed variables statistically no longer deviated from the reference pT_{10}. Both studies show that the $IS_{s.d.}$ decreased with the number of training sessions and intensities, and both studies ultimately use this fact to determine when habituation has taken place. The differences in the speed of habituation between studies, particularly at the trot, is likely to result from the fact that the protracted training used in this study resulted in smaller overall variations per session, and therefore lower threshold values below which a variable had to fall to be judged as habituated. The speed of habituation is also likely to depend on the cumulative amount of time a horse spends on a treadmill in a specific gait. As the walk is a gait that requires more coordinative effort than the trot, this effect is likely to be more pronounced. In the earlier study, the cumulative amount of time spent at the walk was only 45 min (9 x 5 min), which contrast significantly to the 90 min (STG) and 160 min (ETG) of the training groups in our study. It is therefore perhaps not surprising that certain walk parameters in the earlier study never fell below the threshold of habituation.

Lameness diagnostics mainly relies on evaluation of gait asymmetry. It is known that even sound horses have a slight 'natural' asymmetry in their gait variables which ranges within ± 2-6 % [17]. With regard to investigations on a treadmill, the question arises whether this 'natural', or a potentially pathological asymmetry will be enhanced or reduced in horse not fully habituated to treadmill exercise. The use of sign matched asymmetry indices (ASIx) in this study enabled a precise monitoring of the asymmetry during treadmill habituation. At the beginning of the habituation process, horses showed a general tendency towards an enhanced symmetry in both gaits. This finding agrees with our clinical experience where mild lameness are often masked at first visual inspection in non-habituated horses. It is hypothesised that this accentuated left-right symmetry might be the consequence of increased task-oriented concentration of the animal and probably a higher muscle tone in trunk and limbs. Especially gait variables, which potentially might contribute to improve the horse's stability, showed this enhanced symmetry. At walk, this was observable up to 4-5 training sessions in the duration and length of the forelimb stances, in both tripedal and ipsilateral support phases as well as in the second force peaks of both limb pairs. At trot, forelimb and diagonal stance durations remained more symmetrical for up to 4 sessions. However, asymmetry of limb impulses at both gaits and of peak vertical force at trot did not change during the whole habituation process. This is of special importance as peak force asymmetries at trot are the most sensitive parameter in objective lameness assessment [18, 19].

4.3 Differences between the two training regimes

The most notable difference in gait variables between the two training groups was the observation that pT_{10} stride duration at walk was longer in the ETG than in the STG. Consequently, stride rate, stride impulse, forelimb impulse, and stance length showed a group difference as well. At the trot, the same variables showed no significant group difference but a similar tendency No corresponding group difference in size (withers height), which may potentially have caused a difference in stride duration existed, although ETG horses tended to be slightly taller. Considering that the ETG cumulated more training minutes (10 x 30 min vs. 10 x 20 min) it is more probable that these horses attained a more relaxed state of movement at the end of the 10 training tasks. This might explain the pT_{10} value difference and why the initial relative shortening of stride duration was more pronounced in the ETG than in the STG.

Surprisingly, the impact of the different training regimes on the number of training sessions required until habituation occurred was not that important. The specific variables observed in the two training groups adapted quite similarly to the reference, especially at the walk. The hypothesis that the more demanding training of ETG horses would accelerate the process of habituation could not be confirmed. However, the longer training sessions of ETG accelerated the development of a low $IS_{s.d.}$, but only for the commonly more regular trot. A more rapid diminution of $IS_{s.d.}$ of the more variable walking gait did not occur. These obvious coherences regardless of the quite different total treadmill working time after 4 sessions (STG: 80 min; ETG: 120 min) favours the conclusion that the number of exposures to the treadmill is the defining factor for the extent of habituation, and not the duration or complexity of the individual training sessions.

4.4 Heart rate

At the beginning of the first session, HR was distinctly elevated, but after 3 training sessions HR adapted to the reference values pT_{10}. However, at the walk ETG horses showed, despite a break after every training session, persistently elevated HR values at pT_i measurements. This elevated HR level seems to be caused by the longer lasting trotting workload at the end of the extended training exercises. These observations nicely point out that especially at submaximal exercise intensities both mental stress and fatigue may influence the level of HR at a given physical activity level.

5. Conclusions

The actual study showed that after a maximum of 5 training sessions all observed force, limb timing and spatial variables no longer changed with further treadmill experience. Some variables remained steady just after a single session; others required a longer adaptation period with stride duration taking the most time to adapt. The habituation process evolved at a similar rate at walk and trot, although the inter-stride variability at the trot diminished to its final value distinctly faster than that in the walk. Not yet fully habituated horses showed improved symmetry in many variables, in some this phenomenon persisted for up to 5 training sessions; in clinical trials, this might lead to an underestimation of the degree of lameness when assessing a horse on the treadmill. Fortunately, the preferential variables used for lameness objectification such as limb impulses and peak vertical forces reached steady state values after only 3 sessions and the respective symmetry index became stable after just a single treadmill session. A more demanding training regime (ETG) did not accelerate the habituation process by reducing the total number of sessions required. Habituation seems to be more a matter of repetitive exposure to the treadmill and to a lesser extent of the duration and complexity of the training sessions, although longer sessions improved inter-stride regularity at the trot faster. Simple monitoring of heart rate, preferentially at the beginning of each session, was shown to be a valuable way of quantifying the stress to which horses are subjected when copping with the treadmill habituation process.

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